NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF GLUTARALDEHYDE

(CAS NO. 111-30-8)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

September 1999

NTP TR 490

NIH Publication No. 99-3980

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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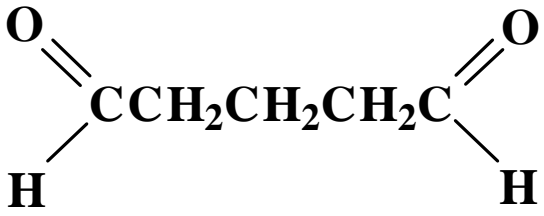
CONTENTS

ABSTRACT



GLUTARALDEHYDE

CAS No. 111-30-8

Chemical Formula: $C_5H_8O_2$ Molecular Weight: 100.13

Synonyms: 1,3-Diformylpropane; glutaral; glutardialdehyde; glutaric dialdehyde; 1,5-pentanedial; 1,5-pentanedione; potentiated acid glutaraldehyde
Trade names: Cidex; Sonacide

Glutaraldehyde is used in large volume in a variety of industries as a disinfectant, preservative, fixative and cross-linking agent, and as a chemical intermediate in the synthesis of pharmaceuticals and pesticides. Glutaraldehyde was nominated by the National Cancer Institute, the Occupational Safety and Health Administration, and the National Institute of Environmental Health Sciences for carcinogenicity studies because of potential occupational exposure. Male and female F344/N rats and B6C3F$_1$ mice were exposed to glutaraldehyde (25% aqueous solution) (approximately 93% pure) by inhalation for 2 years. *In vitro* genetic toxicology studies were conducted in *Salmonella typhimurium*, L5178Y mouse lymphoma cells, and cultured Chinese hamster ovary cells; *in vivo* studies were conducted to measure sex-linked recessive lethal mutations in *Drosophila melanogaster*, chromosomal aberrations and micronucleated erythrocytes in mouse bone marrow, and micronucleated erythrocytes in mouse peripheral blood. The results of 13-week inhalation studies with glutaraldehyde were reported previously (NTP, 1993).

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female F344/N rats were exposed to 0, 250, 500, or 750 ppb glutaraldehyde vapor by inhalation, 6 hours per day, 5 days per week, for 104 weeks. Survival of 500 and 750 ppb female rats was less than that of the chamber controls. Mean body weights of all exposed groups of male rats and 500 and 750 ppb female rats were generally less than those of the chamber controls. Some female rats exposed to 750 ppb were thin to emaciated at the time they were killed moribund. Increased incidences of nonneoplastic nasal lesions occurred primarily within the anterior section of the nose in 500 and 750 ppb rats and to a lesser extent in 250 ppb rats. The more significant lesions included hyperplasia and

inflammation of the squamous and respiratory epithelia and squamous metaplasia of the respiratory epithelium.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 62.5, 125, or 250 ppb glutaraldehyde vapor by inhalation, 6 hours per day, 5 days per week, for 104 weeks. Survival of exposed mice was similar to that of the chamber controls. Mean body weights of female mice exposed to 250 ppb were generally less than those of the chamber controls throughout the study. Incidences of squamous metaplasia of the respiratory epithelium were increased in 250 ppb males and females and 125 ppb females. Incidences of hyaline degeneration of the respiratory epithelium were increased in all exposed groups of females. The incidence of inflammation of the nose was marginally increased in 250 ppb females.

GENETIC TOXICOLOGY

In genetic toxicity studies, glutaraldehyde was mutagenic with and without S9 metabolic activation in *S. typhimurium* strains TA100, TA102, and TA104. Glutaraldehyde was mutagenic in mouse L5178Y lymphoma cells in the absence of S9 and induced sister chromatid exchanges in cultured Chinese hamster ovary cells with and without S9. No increase in chromosomal aberrations was induced by glutaraldehyde in cultured Chinese hamster ovary cells with or without S9 at one laboratory; at another laboratory, chromosomal aberrations were induced in the absence of S9 only. Glutaraldehyde did not induce sex-linked recessive lethal mutations in germ cells of male *D. melanogaster* treated as adults by feeding or injection or treated as larvae by feeding. *In vivo*, glutaraldehyde induced a significant increase in chromosomal aberrations in mouse bone marrow cells 36 hours after a single intraperitoneal injection. In a subset of the 36-hour chromosomal aberrations test, there was a small increase in the number of micronucleated bone marrow polychromatic erythrocytes, which was judged to be equivocal. Additional short-term (3-day) and subchronic (13-week) micronucleus tests in mice, using the intraperitoneal or inhalation routes, respectively, yielded negative results.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity** of glutaraldehyde in male or female F344/N rats exposed to 250, 500, or 750 ppb. There was *no evidence of carcinogenic activity* in male or female B6C3F$_1$ mice exposed to 62.5, 125, or 250 ppb.

Incidences of nonneoplastic lesions of the nose were significantly increased in male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Glutaraldehyde

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in air	Chamber control, 250, 500, or 750 ppb	Chamber control, 250, 500, or 750 ppb	Chamber control, 62.5, 125, or 250 ppb	Chamber control, 62.5, 125, or 250 ppb
Body weights	Exposed groups generally less than chamber controls	500 and 750 ppb groups less than chamber controls	Exposed groups similar to chamber controls	250 ppb group less than chamber controls
Survival rates	12/50, 14/50, 9/50, 6/50	26/50, 31/50, 15/50, 14/50	31/50, 27/50, 40/50, 38/50	34/50, 37/50, 35/50, 32/50
Nonneoplastic effects	Nose: squamous epithelium hyperplasia (3/50, 11/50, 39/50, 48/50); squamous epithelium inflammation (6/50, 17/50, 41/50, 49/50); respiratory epithelium hyperplasia (6/50, 5/50, 17/50, 35/50); respiratory epithelium inflammation (17/50, 10/50, 25/50, 43/50); respiratory epithelium squamous metaplasia (1/50, 2/50, 11/50, 24/50); respiratory epithelium goblet cell hyperplasia (1/50, 0/50, 6/50, 6/50); olfactory epithelium hyaline degeneration (4/50, 8/50, 9/50, 14/50)	Nose: squamous epithelium hyperplasia (3/50, 15/50, 29/50, 45/49); squamous epithelium inflammation (6/50, 26/50, 42/50, 48/49); respiratory epithelium hyperplasia (1/50, 6/50, 15/50, 29/49); respiratory epithelium inflammation (5/50, 9/50, 26/50, 42/49); respiratory epithelium squamous metaplasia (1/50, 1/50, 11/50,16/49); respiratory epithelium goblet cell hyperplasia (1/50, 3/50, 5/50, 8/49); olfactory epithelium hyaline degeneration (4/50, 5/50, 12/50, 15/49)	Nose: respiratory epithelium squamous metaplasia (2/48, 5/50, 6/50, 9/50)	Nose: respiratory epithelium squamous metaplasia (7/50, 11/49, 16/50, 21/50); respiratory epithelium hyaline degeneration (16/50, 35/49, 32/50, 30/50); inflammation (6/50, 7/49, 13/50, 14/50)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Positive in strains TA100, TA102, and TA104 with and without S9			
Mouse lymphoma gene mutations:	Positive without S9			
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells *in vitro*:	Positive with and without S9			
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro*:	Weakly positive without S9			
Mouse bone marrow *in vivo*:	Positive			
Sex-linked recessive lethal mutations				
Drosophila melanogaster:	Negative			
Micronucleated erythrocytes				
Mouse bone marrow *in vivo*:	Equivocal (single-injection protocol); negative (three-injection protocol)			
Mouse peripheral blood *in vivo*:	Negative			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on glutaraldehyde on 30 October 1998 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 30 October 1998, the draft Technical Report on the toxicology and carcinogenesis studies of glutaraldehyde received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. A.P.J.M. van Birgelen, NIEHS, introduced the toxicology and carcinogenesis studies of glutaraldehyde by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *no evidence of carcinogenic activity* in male or female F344/N rats or $B6C3F_1$ mice.

Dr. Belinsky, a principal reviewer, agreed with the proposed conclusions. He commented that, given the high reactivity of glutaraldehyde, it was unlikely that any significant amount reached organs other than the nose. If human exposure is truly restricted to that by inhalation, then the studies are probably adequate; however, if dermal exposure is an issue, other routes should be considered. Dr. van Birgelen said it was plausible that glutaraldehyde does not get beyond the nose, but this was not certain without toxicokinetic data. Dr. Belinsky was also concerned about the inadvertant caloric restriction and asked that this and the issue of tissue distribution be incorporated further in the discussion. Dr. van Birgelen said that decreased incidences of mammary gland and pituitary gland neoplasms may be related to mild decreases in body weight gain in female rats.

Dr. Bus, the second principal reviewer, agreed with the proposed conclusions. He disagreed with the positive findings reported for *Salmonella typhimurium* and sister chromatid exchanges in cultured Chinese hamster ovary cells *in vitro* and chromosomal aberrations in mouse bone marrow cells *in vivo*. He thought that inconsistencies and lack of a dose response supported an equivocal result. Dr. van Birgelen explained how the genetic toxicology results are determined, noting that the results from different laboratories are not combined for a single finding. She said the results for each of the three assays supported a positive finding but agreed that the finding for chromosomal aberrations in mouse bone marrow should be changed to weakly positive. Dr. Bus commented that the section comparing delivered doses of glutaraldehyde to formaldehyde might not be valid in the absence of comparative tissue distribution data.

Dr. Medinsky, the third principal reviewer, agreed with the proposed conclusions. She noted that structure-activity relationships are important in toxicology research to help explain why similar chemicals have different toxic or carcinogenic endpoints. She said the observation that the more reactive glutaraldehyde is deposited primarily in the anterior portion of the nose, whereas formaldehyde is deposited deeper in the respiratory tract, partly explains the marked differences in carcinogenic activity, and that there should be more discussion of this issue.

Ms. J. Kenepp and Ms. S. Sowers, operating-room nurses from New Holland, PA, spoke on behalf of a chemical injury support group, Workers Against Senseless Toxic Exposure (WASTE). Ms. Kenepp stated that hundreds of healthcare professionals had been exposed to glutaraldehyde used as a cold sterilant while not being warned of its toxic effects or being trained in its proper use and disposal. She described health effects that she attributed to glutaraldehyde, including increased sensitivity to the effects of other chemicals. Ms. Sowers mentioned the lack of regulation or control of glutaraldehyde use in the workplace and the need for more research on toxic and carcinogenic effects in humans.

Dr. Bus moved that the Technical Report on glutaraldehyde be accepted with the revisions discussed and the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity*. Dr. Medinsky seconded the motion, which was accepted unanimously with five votes (Drs. Bailer, Bus, Cullen, Hecht, and Medinsky).